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Prediction of synovial inflammation using magnetic resonance imaging in end-stage knee osteoarthritis: a cross-sectional study

Robert GC Riis[1,2], Henrik Gudbergsen[1,3], Ole Simonsen[4], Marius Henriksen[1], Nasir Al-Mashkur[5], Mikkel Eld[5], Kristian Kjær Petersen[6], Olga Kubassova[7], Anne Christine Bay Jensen[8], Janus Damm[2], Henning Bliddal[1], Lars Arendt[6], Mikael Boesen[1,2]

1: The Parker Institute, Dept. of Rheumatology, Copenhagen University Hospital Bispebjerg-Frederiksberg

2: Dept. of Radiology, Copenhagen University Hospital Bispebjerg-Frederiksberg

3: Knowledgecentre for Telemedicine, The Capital Region of Denmark, Hillerød

4: Orthopaedic Surgery Research Unit, Aalborg University Hospital, Aalborg

5: Dept. of Pathology, Aalborg University Hospital, Aalborg

6: Centre for Sensory-Motor Interaction, Aalborg University, Aalborg

7: Image Analysis LTD, London, UK

8: Nordic Bioscience, Herlev, Denmark

Corresponding author:

Mikael Boesen, MD, PhD

Dept. of Radiology,

Copenhagen University Hospital Bispebjerg-Frederiksberg

Copenhagen, Denmark

e-mail: mikael.boesen@gmail.com

Fax: +45 3816 4159

Phone: 45 3816 4158

Introduction

Knee osteoarthritis (KOA) is one the most frequent form of arthritis and a leading cause of physical disability[1;2] imposing substantial socioeconomic costs in a growing elderly and obese population[3;4]. Long-term efficient conservative treatment is scarce eventually leading to total knee arthroplasty (TKA) as the ultimate treatment option. The number of TKA is consequently expected to grow by > 600% from 400,000 in 2003 to 3,500,000 in 2030 in the US alone[5]. KOA has traditionally been considered a degenerative disease ("*wear and tear*") of cartilage and bone[6]. It is however today generally accepted that KOA is a whole joint disease involving all knee joint tissues[7;8]. Clinical, imaging, and biochemical observations indicate that low-grade intra-articular and systemic inflammation may contribute to pain and disease progression[6;9].

Synovitis is defined as inflammation of the synovium and is one of the hallmarks of intra-articular inflammation in KOA. Its role in KOA pathogenesis is not completely clarified but synovitis has been associated with pain, disease severity and increased cartilage degradation[6;8;10-14]. Macroscopically, synovitis can be detected as a thickened, hyperaemic synovium[15]. Nonetheless, histological assessment from synovial biopsies remains the golden standard when assessing synovitis in KOA where inflammatory changes include thickening of the synovial lining and inflammatory infiltrates[16;17].

On magnetic resonance imaging (MRI), synovitis may manifest itself as a thickened and contrast-enhancing synovial membrane and/or indirectly as joint effusion[10;18]. Dynamic contrast-enhanced MRI (DCE-MRI) is a technique based on the sequential acquisition of rapid T1-weighted (T1w) images before and during an IV bolus infusion of Gadolinium contrast (Gd)[19]. Following the injection of the contrast agent, a temporal variation of the MRI signal intensity occurs with the change in signal intensity corresponding to the underlying changes in tissue concentration of contrast agent[20]. With the appropriate software, a time-intensity curve (TIC), i.e. the signal intensity increase over time, can be generated. Quantitative DCE-MRI analysis may be performed by two methodologies: pharmacokinetic or heuristic[21]. Pharmacokinetic analysis approaches use a pre-defined model to characterize the TICs. Most models are based on determining the exchange of contrast agent between blood plasma and extravascular extracellular space (EES) using transfer rate constants such as K^{trans} (volume transfer constant between blood plasma and volume of EES) and K_{ep} (rate constant between EES and blood plasma)[22;23]. Heuristic methods or "curve shape analysis" are based on a voxel-by-voxel analysis. The TICs are extracted from each voxel and assigned to different patterns of contrast uptake, e.g. "no enhancement", "persistent", "plateau" and "wash-out"[24]. Furthermore, parametric maps can be generated from the TIC such as the maximal enhancement (ME) and the initial rate of enhancement (IRE)[25]. In rheumatoid arthritis (RA) the IRE has shown high correlations with histological inflammation of the synovium[18;26]. Thus, the combination of conventional

"static" and dynamic CE-MRI provides a unique ability to investigate all knee joint inflammation related structures, both in regards of morphology and perfusion[10]. However, it remains unknown if and how conventional "static" and dynamic CE-MRI variables relate to histological inflammation in KOA.

Objectives & hypotheses

The aims of this study are: i) to describe the association between MRI, macroscopic and histological assessments of synovitis using correlation analyses and ii) to determine if static and dynamic MRI variables can predict histological synovitis and based on this to develop an MRI-based score/algorithm to be used as a surrogate marker of synovial inflammation. We hypothesize that *MRI based estimates of synovitis are highly and positively correlated (r>0.7) with histopathological findings consistent with synovitis in end-stage KOA.*

Materials and methods

Study design

This study is part of a larger study seeking to investigate the association between synovitis and different assessments of pain (i.e. pain-map, VAS, KOOS etc.) prior to and 1 year after TKA.

In the present cross-sectional study, non-CE, CE- and dynamic CE-MRI of end-stage osteoarthritic knees obtained before surgery will be analysed to quantify the extent of synovitis, using perfusion variables as surrogate markers of inflammation, and correlated with microscopic and macroscopic assessments of synovitis obtained from tissue samples during surgery.

All data have been collected, and this protocol represents a pre-specification of the procedures and processes necessary to establish the criterion validity of MRI-variables in assessing synovitis in knee osteoarthritis.

Study population

Participants were recruited from the Department of Orthopaedic Surgery, Aalborg University Hospital, Denmark, upon referral to TKA. Eligibility criteria were as follows: age > 18 years; symptomatic, primary (idiopathic) KOA according to the American College of Rheumatology criteria, radiographically verified[27]. In case of bilateral KOA, the knee scheduled for arthroplasty was defined as the target knee. Subjects were excluded if any of the following criteria was present: pregnancy or planned pregnancy; mental impairment or insufficient Danish skills precluding an informed consent; contraindications for MRI (e.g. magnetic implants). Furthermore, DCE-MRI was not performed if the patient had an estimated glomerular filtration rate (eGFR) < 60 ml/min/1.73m^2, in accordance with The European Medicines Agency's guidelines on the administration of IV

Gadolinium-containing contrast agents[28]. The study was approved by the local ethical committee (N-20110031) and conducted according to the Helsinki declaration as revised in 2000. All participants gave their oral and written informed consent.

Sample size

As this is a descriptive study and no similar studies have been published prior to this, the power calculations are based on the confidence intervals (CI) of the correlations: with a sample size of 60, correlation coefficients of 0.0 (no correlation), 0.4 (moderate correlation) and 0.8 (high correlation) will result in 95% CI of -0.26-0.26, 0.15-0.60 and 0.68-0.88 respectively. This is accepted and regarded sufficient for the planned regression analyses.

MRI protocol

MRI of the target knee was performed on a Philips Intera® 1.5 Tesla system. The subjects were scanned in the supine position using a SENSE flex M coil. The following MRI-protocol was used: 3 plane GRE (gradient echo) scout (matrix 256x256 mm, FOV (field of view) 300 mm, TE 7.1 ms, TR 13.4 ms, ST (slice thickness) 8mm); sagittal T1w TSE (turbo spin echo) (matrix res 1024x1024, FOV 160 mm, TE 18 ms, TR 515 ms, ST 3 mm); sagittal PDw (proton density weighted) TSE (matrix 512x512 mm, FOV 160 TE 30 ms, TR 2639 ms, ST 3 mm); sagittal PDw SPIR (matrix 512x512, FOV 160 mm, TE 30 ms, TR 2686 ms, ST 3 mm); axial PDw SPIR (matrix 512x512, FOV 160 mm, TE 30 ms, TR 3000 ms, ST 3 mm); coronal PDw SPIR (matrix 512x512, FOV 160 mm, TE 25 ms, TR 1500 ms, ST 3 mm). Just prior to and simultaneously with the IV injection of 0.1 ml/kg body weight Gadolinium contrast (Gadovist®) using a power injector (2 ml/s), a sequential sagittal DCE-MRI T1w sequence was performed (matrix 352x352, FOV 180 mm, TE 4.6 ms, TR 8.3 ms, ST 8 mm, FA (flip angle) 12°). Following this the static TSE T1w sequence was repeated (matrix res 1024x1024, FOV 160 mm, TE 18 ms, TR 515 ms, ST 3 mm). Total scan time varied between 17-20 minutes.

Image analysis

A resident and PhD fellow in MSK radiology (RR) performed all the MRI assessments, supervised by a senior consultant in MSK radiology (MB). Both were blinded to the histological and macroscopic data. The DCE-MRIs were analysed using Dynamika® Enterprise v. 3.2.1 (www.imageanalysis.org.uk) after application of motion

correction thus improving the signal-to-noise ratio[29], regions of interest (ROIs), were drawn around the synovium covering the suprapatellar pouch (incl. the biopsy sites) and the medial and lateral recesses where possible on the sagittal DCE-MRI slices. These ROIs were then collapsed into a single VOI (volume of interest) from which a set of perfusion variables was extracted (VOI_{total}). Any effusion in the recesses was included in the ROIs, but as effusion-voxels are not perfused but only enhancing secondary to diffusion, they are automatically classified as "no enhancement" by the software; thus the perfusion variables extracted from the VOI, i.e. the means of the IRE and ME, represent the perfusion of the synovium alone and not the effusion. Osirix® v. 5.7.1 was used to confirm the anatomical boundaries of the synovium and score the static images: in the MOAKS (MRI in OA Knee Score) synovitis is scored semi-quantitavely as "effusion-synovitis" and "Hoffa-synovitis"[30]: effusion-synovitis is the combination of effusion and synovitis defined as the hyperintense signal in the suprapatellar recess on fluid sensitive sequences (0: physiological amount, 1: small – fluid continuous with the retropatellar space, 2: medium – with slight convexity of the suprapatellar recess, 3: large – evidence of capsular distension). Hoffa-synovitis is defined as the extent of hyperintense signal changes in the IPFP on mid-sagittal fluid-sensitive sequences (0: normal, 1: mild, 2: moderate, 3: severe). In both cases the sagittal and reconstructed axial pre-contrast 3D PDw were used as recommend in the MOAKS[30]. The two scores were then collapsed into one single "*MOAKS_Synovitis"* score (0-6). Since the MRI protocol included CE sequences and thus enabled us to clearly differentiate effusion from synovitis[10], we chose to score effusion according to the BLOKS (Boston-Leeds OA Knee Score) (0: physiological amount, 1: small – fluid continuous with the retropatellar space, 2: medium – with slight convexity of the suprapatellar recess, 3: large – evidence of capsular distension)[31] using the sagittal post-contrast T1w TSE. We additionally assessed synovitis according to Guermazi et al.[32] based on the thickness (0: < 2 mm; 1: 2-4 mm; 2: > 4 mm) of the synovium in 11 different locations in the knee (suprapatellar, infrapatellar, intercondylar, medial and lateral recess, adjacent to ACL/PCL, perimeniscal (medial/lateral), Baker cysts and around loose bodies), thereby generating a whole-knee synovitis score ("*CE Synovitis*"), ranging from 0 to 22. As the post-contrast MRI sequence was a T1w TSE without fat-suppression, the enhancing synovium could not clearly be differentiated from adipose tissue. This problem was encompassed by using the *subtraction* and *fusion* functions in Osirix®, i.e. the pre-contrast sagittal T1w TSE was subtracted from (respectively fused with) the sagittal post-contrast T1w TSE (Figure 1) and synovitis was scored as previously described.

Figure 1. Subtraction (C) and Fusion (D) images of pre-contrast (A) and post-contrast (B) T1w images. Arrows are marking the enhancing synovium.

Histological protocol and assessment

Synovial excision biopsies of approximately 1x1 cm were taken intraoperatively by OS from the following locations: i) suprapatellar pouch anteriorly and ii) posteriorly, iii) medial and iv) lateral recesses, v) most severe synovitis macroscopically and vi) most severe synovitis on MRI. The biopsies were immediately immersed in a formalin solution, numerated and stored until embedment in paraffin. From each biopsy one slice will be obtained using a 3 μm microtome and stained with hematoxylin-eosin (HE). A resident in pathology (NA) will perform all the histological assessments supervised by a senior consultant in pathology (ME). Both are blinded to the imaging and macroscopic data. The slices will be assessed and scored semiquantitavely 0-3 for the following histopathological qualities according to Krenn et al.[17]: i) hyperplasia/enlargement of the synovial lining cell layer (0: absent; 1: slight (2-3 cell layers); 2: moderate (4-5 cell layers); 3: strong (≥ 6 cell layers)), ii) inflammatory infiltration (0: absent; 1: slight (diffusely located single cells and small perivascular aggregates of lymphocytes and/or plasma cells), 2: moderate (perivascular and/or superficial lymphatic aggregates); 3: strong (lymphatic follicles with germinal centre and/or confluent subsynovial lymphatic infiltration)) and iii) activation of synovial stroma (0: absent; 1: slight (low cellularity with slight edema and fibrosis with some fibroblasts); 2:

moderate (moderate cellularity with moderate density of fibroblasts and endothelial cells); 3: strong (high cellularity with dense distribution of fibroblasts and endothelial cells, giant cells are abundant)). For each patient, an average grade will be calculated for each feature. The three averages are then summed, creating a total histology score ranging from 0-9.

Macroscopic assessment

The synovium is assessed macroscopically intraoperatively by OS according to a validated system as proposed by af Klint et al. [15]. Three parameters (hypertrophy, vascularity and synovitis) are scored 0-4 and summed, creating a total macroscopic score ranging from 0-12.

Outcome measures

The DCE-MRI variables are as follows:

- *Nvoxel*, the number of voxels with plateau or washout patterns, i.e. the most perfused voxels
- *Nclassified*, the number of voxels either classified as persistent, plateau or washout
- *Nvoxel%*, the proportion of highly perfused voxels (Nvoxel) over the total number of classified voxels (persistent, plateau, washout)
- *IRE*, the initial rate of enhancement, i.e. the upslope on the TIC measured as the relative increase in the signal intensity per second (%/s)
- *ME*, the maximal enhancement (ME), i.e. the highest signal intensity value relative to the baseline intensity. In general the higher an IRE and ME, the higher a perfusion.
- *IRExNvoxel* and *MExNvoxel*, the initial rate of enhancement (IRE) and maximum enhancement (ME) multiplied by Nvoxel. As a voxel represents a volume, N_{voxel} can be regarded as the volume of the highest perfused synovium whereas the IRE and ME represent the degree of perfusion. By multiplying N_{voxel} with the mean IRE and mean ME respectively, we create two composite variables reflecting both the volume and degree of perfusion.
- *IRExME*, the mean initial rate of enhancement multiplied by the mean maximum enhancement). We furthermore chose to multiply the mean IRE and mean ME, creating IRExME, as we believe that these two parameters are the most defining in characterising the perfusion profile of the voxels.
- *AUC*, the area under the curve is the area under the TIC,

- *Tonset* is the time of onset (in seconds) from the beginning of DCE-MRI sequence to the onset of contrast-enhancement. The lower a Tonset, the higher a perfusion. A high value of Tonset, combined with high ME and IRE values are an indicator of inflammation, while a low Tonset coupled with high ME and IRE values indicate that the area is a blood vessel.
- *Twashout*, the time (in seconds) to washout/decrease in contrast-enhancement. A low Twashout coupled with high ME and IRE values are indices of a blood vessel.
- *IRW*, the initial of washout, i.e. the downslope on the TIC in %/s.

N_{voxel}, Nvoxel%, IRExNvoxel, MExNvoxel and AUC are all variables that have been used in previous studies using perfusion variables obtained from in both inflammatory arthritis and KOA DCE-MRI[26;33-37].

Furthermore the following pharmacokinetic variables will be extracted from the DCE-MRI data:

- *Ktrans*, is the volume transfer coefficient for passage of contrast agent from the blood vessel to the extracellular space over time (min^{-1}) and thus a measure of capillary permeability (higher values indicating higher permeability as seen in inflammation).
- *Ve*, the proportion (between 0 and 1) of extra-vascular, extra-cellular space in the ROI
- *iAUGC60*, the initial area under the gadolinium curve over 60 seconds.

The static MRI variables include:

- *MOAKS_Synovitis* (the sum of Hoffa-synovitis and effusion-synovitis according to the MOAKS[30])
- *BLOKS_Effusion* (effusion according to the BLOKS[31]) and
- *CE_Synovitis* (the whole-knee synovitis score according to Guermazi et al.[32]).

Secondary laboratory clinimetrics include:

- Plasma levels of C-reactive protein (CRP),
- Body mass index (BMI) and
- Kellgren-Lawrence (KL) grades[38] of the target knee.

The microscopic variables consist of a sum score (0-9) of the following subscales, each scored 0-3 according to Krenn et al.[17]: i) hyperplasia/enlargement of the synovial lining cell layer, ii) inflammatory infiltration and iii) activation of synovial stroma.

The macroscopic variable consist of a sum score (0-12) of the three subscales (hypertrophy, vascularity and synovitis), each scored 0-4.

Statistics

The knee joint will be assessed as a whole and the variables needed for this approach will be: i) the perfusion variables from VOI_{total}, ii) the total-histology score, iii) the total-macroscopic score, iv) CE_Synovitis, v) the MOAKS_Synovitis, and vi) BLOKS_Effusion.

All analyses will be performed using SAS/SPSS software. Two-sided statistical test will be used and a p-value < 0.05 will be considered statistically significant. The analyses will be pre-specified in a statistical analysis plan made available online from www.parkerinst.dk. All analyses will be conducted on the *existing case* population (no imputation for missing data).

Spearman's correlation will be used to evaluate bivariate associations between all basic characteristics, MRI, microscopic and macroscopic variables (Table 2). In order to compensate for the issue of multiple testing, only correlations coefficients ≥ 0.70 will be regarded as statistically significant.

This will be followed by multiple regression analyses with the basic characteristics and MRI variables as predictors and the histology score as outcome variable. As contrast-enhanced MRI is not routinely performed in KOA, we choose to perform three different multiple regression analyses with different sets of predictors in order to increase the feasibility and clinical applicability:

- Model 1 will include all basic characteristics and static, non-CE MRI variables (i.e. MOAKS_Synovitis)
- Model 2 will include the aforementioned variables from model 1 and the static, CE-MRI variables
- Model 3 will include the variables from the two previous models and all DCE-MRI variables

As especially model 3 will include several variables (> 20), we intend to do a factor analysis prior to the multiple regression analyses. The independent variables may be transformed (e.g. √, log, ln) in order to improve the model fit and the regression analyses may be repeated using transformed variables. In case of transformation only the transformed variable will be included, i.e. each variable can only be included once, either in its transformed or raw form.

In all three cases, multiple regression analyses will be performed with the intention to find the subset of independent variables (MRI-variables) that best predict the dependent variable (histology) by linear regression

in our sample in terms of the largest adjusted R^2. The constants and regression coefficients of final model will represent MRI-based scores/algorithms to be used as a surrogate marker of synovial inflammation.

Results

Participant flow

Figure 2. Trial profile

Baseline data

Table 1. Demographic, imaging, histological and macroscopic variables

Basic characteristics	
Female, no. (%)	0-100
Age (years)	18-90
BMI (kg/m^2)	18-40
Symptoms duration (years)	1-50
CRP (mg/l)	0-100
Radiographs	
Kellgren-Lawrence	0-4
Non CE-MRI†	
Hoffa-synovitis	0-3
Effusion-synovitis	0-3
Total synovitis	0-6
CE-MRI	
Total synovitis*	0-22
BLOKS Effusion≠	0-3
DCE-MRI	
Nvoxel	0-50000
Nclassified	0-100000
Nvoxel%	0-100
IRE	0-100
ME	0-100
IRExME	0-100000
IRExNvoxel	0-100000
MExNvoxel	0-100000
AUC	0-100000
Tonset	0-1000
Twashout	0-1000
IRW	0-100
Ktrans	0-1000
Ve	0-1000
iAUGC60	0-10000
Histology¶	
Synovial lining, mean	0-3
Stroma, mean	0-3
Infiltration, mean	0-3
Total, sum of means	0-9
Macroscopic~	
Hypertrophy	0-4
Vascularity	0-4
Synovitis	0-4
Total	0-12

†according to the MOAKS[30]; *according to Guermazi et al.[32]; ≠according to the BLOKS[31]; ¶according to Krenn et al.[17]; ~according to af Klint et al.[15]

Outcomes

Table 2 – Spearman's rank correction matrix of all variables

	Histology	Macroscopic	DCE-MRI														CE-MRI		non CE-MRI	Basis characteristics					
	Synovitis micro	Synovitis macro	Nvoxel	Nclassified	Nvoxel%	IRE	ME	IRExME	IRExNvoxel	MExNvoxel	AUC	Tonset	Twshout	IRW	Ktrans	Ve	iIAUGC60	CE_Synovitis	BLOKS_Effusion	MOAKS_Synovitis	Age	Sex	Symptoms duration	BMI	CRP	KL
Synovitis micro	1.0																									
Synovitis macro		1.0																								
Nvoxel			1.0																							
Nclassified				1.0																						
Nvoxel%					1.0																					
IRE						1.0																				
ME							1.0																			
IRExME								1.0																		
IRExNvoxel									1.0																	
MExNvoxel										1.0																
AUC											1.0															
Tonset												1.0														
Twashout													1.0													
IRW														1.0												
Ktrans															1.0											
Ve																1.0										
IAUGC60																	1.0									
CE_Synovitis																		1.0								
BLOKS_Effusion																			1.0							
MOAKS_Synovitis																				1.0						
Age																					1.0					
Sex																						1.0				
Symptoms duration																							1.0			
BMI																								1.0		
CRP																									1.0	
KL																										1.0

Table 3. Factor analyses

Table 4. Regression analyses

Author contribution

RR will perform all MRI analyses, contribute to the study design, statistical design and analysis, interpretation of the data, draft and revise the manuscript, and approve the final version. *HG* will contribute to the interpretation of the data, revise the manuscript, and approve the final version. *OS* performed all TKA (including synovial sampling) and will contribute to the study design, interpretation of the data, revise the manuscript, and approve the final version. *NA* will perform all histological analyses and contribute to the interpretation of the data, revise the manuscript, and approve the final version. *MH* will contribute to the study design, statistical design and analysis, interpretation of the data, revision of the manuscript, and approve the final version. *ME* will contribute to the study design, histological supervision, and interpretation of the data, revise the manuscript, and approve the final version. *KKP* will contribute to the study design, the interpretation of the data, revise the manuscript, and approve the final version. *OK* will contribute to the study design, the interpretation of the data, revise the manuscript, and approve the final version. *JD* will contribute to the interpretation of the data, revise the manuscript, and approve the final version. *HB* will contribute to the study design, the interpretation of the data, revise the manuscript, and approve the final version. *LA* will contribute to the study design, the interpretation of the data, revise the manuscript, and approve the final version. *MB* will contribute to the study design, radiological supervision, and interpretation of the data, revise the manuscript, and approve the final version. All the authors approved the study protocol and will have access to the data and analyses. *RR* takes responsibility for the integrity of the work as a whole, from inception to final draft.

Reference List

(1) Johnson VL, Hunter DJ. The epidemiology of osteoarthritis. *Best Pract Res Clin Rheumatol* 2014; 28(1):5-15.

(2) Lawrence RC, Felson DT, Helmick CG, Arnold LM, Choi H, Deyo RA et al. Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part II. *Arthritis Rheum* 2008; 58(1):26-35.

(3) Hunter DJ, Schofield D, Callander E. The individual and socioeconomic impact of osteoarthritis. *Nat Rev Rheumatol* 2014; 10(7):437-441.

(4) Wang YC, McPherson K, Marsh T, Gortmaker SL, Brown M. Health and economic burden of the projected obesity trends in the USA and the UK. *Lancet* 2011; 378(9793):815-825.

(5) Kurtz S, Ong K, Lau E, Mowat F, Halpern M. Projections of primary and revision hip and knee arthroplasty in the United States from 2005 to 2030. *J Bone Joint Surg Am* 2007; 89(4):780-785.

(6) Berenbaum F. Osteoarthritis as an inflammatory disease (osteoarthritis is not osteoarthrosis!). *Osteoarthritis Cartilage* 2013; 21(1):16-21.

(7) Conaghan PG, Kloppenburg M, Schett G, Bijlsma JW. Osteoarthritis research priorities: a report from a EULAR ad hoc expert committee. *Ann Rheum Dis* 2014; 73(8):1442-1445.

(8) Hunter DJ, Zhang W, Conaghan PG, Hirko K, Menashe L, Li L et al. Systematic review of the concurrent and predictive validity of MRI biomarkers in OA. *Osteoarthritis Cartilage* 2011; 19(5):557-588.

(9) Sofat N, Ejindu V, Kiely P. What makes osteoarthritis painful? The evidence for local and central pain processing. *Rheumatology (Oxford)* 2011; 50(12):2157-2165.

(10) Hayashi D, Roemer FW, Katur A, Felson DT, Yang SO, Alomran F et al. Imaging of synovitis in osteoarthritis: current status and outlook. *Semin Arthritis Rheum* 2011; 41(2):116-130.

(11) Hill CL, Gale DG, Chaisson CE, Skinner K, Kazis L, Gale ME et al. Knee effusions, popliteal cysts, and synovial thickening: association with knee pain in osteoarthritis. *J Rheumatol* 2001; 28(6):1330-1337.

(12) Hill CL, Hunter DJ, Niu J, Clancy M, Guermazi A, Genant H et al. Synovitis detected on magnetic resonance imaging and its relation to pain and cartilage loss in knee osteoarthritis. *Ann Rheum Dis* 2007; 66(12):1599-1603.

(13) Yusuf E, Kortekaas MC, Watt I, Huizinga TW, Kloppenburg M. Do knee abnormalities visualised on MRI explain knee pain in knee osteoarthritis? A systematic review. *Ann Rheum Dis* 2011; 70(1):60-67.

(14) Zhang Y, Nevitt M, Niu J, Lewis C, Torner J, Guermazi A et al. Fluctuation of knee pain and changes in bone marrow lesions, effusions, and synovitis on magnetic resonance imaging. *Arthritis Rheum* 2011; 63(3):691-699.

(15) af KE, Catrina AI, Matt P, Neregrad P, Lampa J, Ulfgren AK et al. Evaluation of arthroscopy and macroscopic scoring. *Arthritis Res Ther* 2009; 11(3):R81.

(16) de Lange-Brokaar BJ, Ioan-Facsinay A, van Osch GJ, Zuurmond AM, Schoones J, Toes RE et al. Synovial inflammation, immune cells and their cytokines in osteoarthritis: a review. *Osteoarthritis Cartilage* 2012; 20(12):1484-1499.

(17) Krenn V, Morawietz L, Haupl T, Neidel J, Petersen I, Konig A. Grading of chronic synovitis--a histopathological grading system for molecular and diagnostic pathology. *Pathol Res Pract* 2002; 198(5):317-325.

(18) Loeuille D, Rat AC, Goebel JC, Champigneulle J, Blum A, Netter P et al. Magnetic resonance imaging in osteoarthritis: which method best reflects synovial membrane inflammation? Correlations with clinical, macroscopic and microscopic features. *Osteoarthritis Cartilage* 2009; 17(9):1186-1192.

(19) Verstraete KL, Van der Woude HJ, Hogendoorn PC, De-Deene Y, Kunnen M, Bloem JL. Dynamic contrast-enhanced MR imaging of musculoskeletal tumors: basic principles and clinical applications. *J Magn Reson Imaging* 1996; 6(2):311-321.

(20) Bliddal H, Boesen M, Christensen R, Kubassova O, Torp-Pedersen S. Imaging as a follow-up tool in clinical trials and clinical practice. *Best Pract Res Clin Rheumatol* 2008; 22(6):1109-1126.

(21) Kubassova O, Boesen M, Peloschek P, Langs G, Cimmino MA, Bliddal H et al. Quantifying disease activity and damage by imaging in rheumatoid arthritis and osteoarthritis. *Ann N Y Acad Sci* 2009; 1154:207-238.

(22) Paldino MJ, Barboriak DP. Fundamentals of quantitative dynamic contrast-enhanced MR imaging. *Magn Reson Imaging Clin N Am* 2009; 17(2):277-289.

(23) Tofts PS, Brix G, Buckley DL, Evelhoch JL, Henderson E, Knopp MV et al. Estimating kinetic parameters from dynamic contrast-enhanced T(1)-weighted MRI of a diffusable tracer: standardized quantities and symbols. *J Magn Reson Imaging* 1999; 10(3):223-232.

(24) Kubassova OA, Boyle RD, Radjenovic A. Quantitative analysis of dynamic contrast-enhanced MRI datasets of the metacarpophalangeal joints. *Acad Radiol* 2007; 14(10):1189-1200.

(25) Boesen M, Kubassova O, Parodi M, Bliddal H, Innocenti S, Garlaschi G et al. Comparison of the manual and computer-aided techniques for evaluation of wrist synovitis using dynamic contrast-enhanced MRI on a dedicated scanner. *Eur J Radiol* 2011; 77(2):202-206.

(26) Axelsen MB, Stoltenberg M, Poggenborg RP, Kubassova O, Boesen M, Bliddal H et al. Dynamic gadolinium-enhanced magnetic resonance imaging allows accurate assessment of the synovial inflammatory activity in rheumatoid arthritis knee joints: a comparison with synovial histology. *Scand J Rheumatol* 2012; 41(2):89-94.

(27) Altman R, Asch E, Bloch D, Bole G, Borenstein D, Brandt K et al. Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and

Therapeutic Criteria Committee of the American Rheumatism Association. *Arthritis Rheum* 1986; 29(8):1039-1049.

(28) Kirchin MA, Runge VM. Contrast agents for magnetic resonance imaging: safety update. *Top Magn Reson Imaging* 2003; 14(5):426-435.

(29) Boesen M, Kubassova O, Bliddal H, Ostergaard M, Danneskiold-Samsoe B, Cimmino MA. Automatic computer aided quantification of synovitis using Dynamic MRI and the impact of movement correction on signal to noise (SNR) and region of interest (ROI) analysis. Arthritis Rheum. 60 Supplement 10:773. 2009. Ref Type: Abstract

(30) Hunter DJ, Guermazi A, Lo GH, Grainger AJ, Conaghan PG, Boudreau RM et al. Evolution of semi-quantitative whole joint assessment of knee OA: MOAKS (MRI Osteoarthritis Knee Score). *Osteoarthritis Cartilage* 2011; 19(8):990-1002.

(31) Hunter DJ, Lo GH, Gale D, Grainger AJ, Guermazi A, Conaghan PG. The reliability of a new scoring system for knee osteoarthritis MRI and the validity of bone marrow lesion assessment: BLOKS (Boston Leeds Osteoarthritis Knee Score). *Ann Rheum Dis* 2008; 67(2):206-211.

(32) Guermazi A, Roemer FW, Hayashi D, Crema MD, Niu J, Zhang Y et al. Assessment of synovitis with contrast-enhanced MRI using a whole-joint semiquantitative scoring system in people with, or at high risk of, knee osteoarthritis: the MOST study. *Ann Rheum Dis* 2011; 70(5):805-811.

(33) Axelsen M, Poggenborg R, Stoltenberg M, Kubassova O, Boesen M, Horslev-Petersen K et al. Reliability and responsiveness of dynamic contrast-enhanced magnetic resonance imaging in rheumatoid arthritis. *Scand J Rheumatol* 2012.

(34) Ballegaard C, Riis RG, Bliddal H, Christensen R, Henriksen M, Bartels EM et al. Knee pain and inflammation in the infrapatellar fat pad estimated by conventional and dynamic contrast-enhanced magnetic resonance imaging in obese patients with osteoarthritis: A cross-sectional study. *Osteoarthritis Cartilage* 2014; 22(7):933-940.

(35) Boesen M, Kubassova O, Bouert R, Axelsen MB, Ostergaard M, Cimmino MA et al. Correlation between computer-aided dynamic gadolinium-enhanced MRI assessment of inflammation and semi-quantitative synovitis and bone marrow oedema scores of the wrist in patients with rheumatoid arthritis--a cohort study. *Rheumatology (Oxford)* 2012; 51(1):134-143.

(36) Cimmino MA, Parodi M, Zampogna G, Boesen M, Kubassova O, Barbieri F et al. Dynamic contrast-enhanced, extremity-dedicated MRI identifies synovitis changes in the follow-up of rheumatoid arthritis patients treated with rituximab. *Clin Exp Rheumatol* 2014; 32(5):647-652.

(37) Bandak E, Boesen M, Bliddal H, Riis RG, Gudbergsen H, Henriksen M. Associations between muscle perfusion and symptoms in knee osteoarthritis: a cross sectional study. *Osteoarthritis Cartilage* 2015.

(38) Kellgren JH, Lawrence JS. Radiological assessment of osteo-arthrosis. *Ann Rheum Dis* 1957; 16(4):494-502.